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Published online: 24 Mar 2016.

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Administration of *Aloe arborescens* homogenate to cattle: interaction with rumen fermentation and gut absorption of aloin

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ABSTRACT

Aloe has long been used as a traditional medicine for its numerous beneficial properties, which are mainly ascribed to β-polysaccharides and phenolic compounds including anthraquinones, anthrones and chromones. However, few studies on large animals are currently available. The effect of whole leaf *Aloe arborescens* homogenate on the *in vitro* rumen fermentative processes was tested using alfalfa hay and barley meal as substrates. The Aloe homogeneate was added at three different concentrations (0.4, 2.0 and $10.0 \, g \, L^{-1}$ of fermentation liquid). The same homogenate was dosed (200 g) orally and through the rumen cannula to three rumen cannulated heifers and orally to six lactating dairy cows to measure the rumen degradation of aloin and the transfer of aloin from the gut into the blood, respectively. The Aloe homogenate did not affect *in vitro* rumen fermentations and feed digestibility. The administration of Aloe homogenate did not negatively affect animal feed intake and health neither on the cannulated heifers nor on the dairy cows. Aloin underwent a rapid degradation in the rumen milieu, and became undetectable 2 h after oral dosage. However, when Aloe homogenate was administered to dairy cows, aloin appeared in blood as early as 2 h after administration, reached a maximum after 4 h ($6.2 \pm 5.8 \, \mu g \, L^{-1}$) and progressively decreased thereafter. These results suggest that Aloe compounds can be absorbed into the blood and encourage the study of Aloe as a potential nutraceutical in ruminants. Further studies should determine the most effective *in vivo* dosage.

Introduction

Aloe spp. is widely used in traditional medicine due to their therapeutic properties (Grindlay & Reynolds 1986). The most important species are *Aloe barbadensis* Mill. (Commonly known as *Aloe vera*) and *Aloe arborescens* Mill. (Liao et al. 2006). The inner leaf gel of Aloe species contains typical polysaccharides, such as acemannans and aloerides, as well as pectins which exhibit wound-healing, anti-inflammatory, immune-stimulant, anti-bacterial and antioxidant properties (Hamman 2008; Pugh et al. 2001). The whole leaves are also rich in secondary phenolic metabolites including anthraquinones, anthrones and chromones, including their glycosides derivatives. The most well known is barbaloin (10-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9-10H-anthracenone), a mixture of the two diastereoisomers aloin A and aloin B (Fanali et al. 2010). These components are concentrated in the green rind of the leaves and appear to have several activities (e.g. antioxidant, antimicrobial and anti-inflammatory; Gutterman & Chauser-Volfson 2000; Lucini et al. 2015). Recently, the

activity of the anthraquinones against several cancer cells lines has been reviewed (Lucini et al. 2012).

The biological properties of Aloe products are affected by several factors, and in particular by the portion of the plant actually used, as some of the active compounds are located in the derma (e.g. glycoside anthraquinones), whereas some others are in the inner gel (e.g. acemannans and glucomannans) (Colla et al. 2013; Lucini et al. 2013). Other important factors for biological activities concern extraction technique, processing and storage conditions, the leaf portion considered and the stage of development and the health status of the plants (Amruta & Purushottam 2007; Romani et al. 2008; Pellizzoni et al. 2012). In particular, Aloe leaves are reported to lose their biological activity starting from 6 h after harvest at room temperature starting (Ramachandra & Srinivasa Rao 2008). The age of the plants plays an important role: Hu et al. (2003) reported that leaves from three years old *Aloe barbadensis* plants contained significantly higher levels of polysaccharides

and flavonoids than leaves of two and four years old plants.

Scientific information regarding the effect on the animal metabolism from Aloe spp. whole plant or from some of its components is scanty and only a recent cause of interest (Gutterman & Chauser-Volfson 2000; Beppu et al. 2006; Shin et al. 2011; Misawa et al. 2012). No information is available on the fate of active components from Aloe spp. in the digestive system of ruminants, although the rate of rumen passage and the absorption kinetics of the major components in the gut could play a significant role in Aloe spp. activity. Finally, interference with the rumen metabolism and microbial degradation in ruminants by rumen microbiota has rarely been investigated (Calabrò et al. 2013). This study aimed to test the effects of whole leaf homogenate of Aloe arborescens on in vitro rumen fermentation (experiment 1), the in vivo rumen degradation of aloin (experiment 2) and to evaluate the absorption of aloin into the blood of lactating dairy cows after oral administration (experiment 3).

Materials and methods

This study complied in accordance with the Italian and European laws on animal experimentation and ethics.

Preparation of homogenate from Aloe arborescens

Three years old Aloe arborescens plants and grown under a plastic greenhouse (Dester Gardens, Crociale di Manerba del Garda, BS, Italy) were used. In previous protocols (Lucini et al. 2013) this type of plants demonstrated a high and steady anthraquinones content.

The whole leaves of the plants were cut, homogenised by a commercial vegetable cutter (model R6, Robot Coupe, Vincennes Cedex, France). Homogenate was divided into 200 g aliquots that were immediately frozen and stored at $-20\,^{\circ}$C until used. This storage method has been demonstrated to guarantee a high stability of the homogenate without any additive (Pellizzoni et al. 2011).

The homogenate of Aloe was analysed for DM (method AOAC 930.15, AOAC 2005), CP (method AOAC 990.03, AOAC 2005), ether extract (method AOAC 920.39, AOAC 2005) and ash (method AOAC 942.05, AOAC 2005) content. Ash-free Neutral Detergent Fiber (NDFom) was determined according to (Van Soest et al. 1991), omitting alfa amylase and sodium sulphite. Aloin and aloe-emodin, which are considered to be the main secondary phenolic metabolites of Aloe (Fanali et al. 2010), were determined as described below.

Experiment 1: effects of the Aloe arborescens homogenate on in vitro rumen fermentations

All animals used in this study were cared for in accordance with the 116/92 guidelines under Italian law on animal welfare for experimental animals. Samples were fermented in vitro according to Mauricio et al. (1999), with modifications as described in Ahmed et al. (2013), to measure in vitro gas production, dry matter digestibility (IVDMD) and production of VFA (Table 1).

Gas production techniques were based on measurement of the headspace gas pressure in serum vials. Two substrates samples (alfalfa hay or barley meal) were weighed (0.5 g) in 125 mL serum vials, followed by addition of 30 mL of medium (Goering & Van Soest, 1970) and 10 mL of rumen fluid, previously filtered through a synthetic cheese cloth under a flux of O_2-free CO2. Aloe homogenate was diluted in the medium at concentrations of 0.4, 2.0 and 10.0 g L^{-1} of fermentation liquid. Ruminal fluid was collected into thermos vials 6 h after the morning feeding from three rumen cannulated Italian Friesian heifers fed twice daily ration consisting of grass hay (8 kg), concentrate (1 kg) and a vitamin and mineral supplement. Vials were sealed with butyl rubber stoppers and aluminium

Table 1. Effects of Aloe arborescens homogenate on in vitro ruminal fermentation.

Item		Time		Substrate		Dose				MSE	p value		
		8	24	Alfalfa	Barley	0	20	100	500		T	S	D
DMD	g kg^{-1}	533	669	465	737	570	588	619	626	700.45	<0.001	<0.001	0.24
pH		6.78	6.74	6.73	6.78	6.75	6.76	6.76	6.77	0.0120	0.11	0.07	0.96
GP	ml g^{-1} OM	132	242	163	212	187	187	188	187	65.911	<0.001	<0.001	0.98
Total VFA	mM	64.9	102.2	80.8	86.3	81.5	83.6	84.1	84.9	13.6764	<0.001	<0.001	0.10
Acetate	%	59.04	57.37	65.46	50.95	58.69	58.29	58.23	57.61	51.4789	0.36	<0.001	0.99
Propionate	%	31.87	26.09	26.10	31.86	28.69	28.92	28.93	29.39	19.2930	<0.001	<0.001	0.99
Butyrirate	%	8.28	11.63	6.02	13.89	9.70	9.92	9.96	10.25	8.0231	<0.001	<0.001	0.98
Isobutyrate	%	0.11	1.19	0.76	0.54	0.67	0.65	0.65	0.63	0.0128	<0.001	<0.001	0.67
Valerate	%	0.47	2.78	1.13	2.12	1.64	1.64	1.64	1.59	0.0177	<0.001	<0.001	0.77
Isovalerate	%	0.23	1.96	0.93	1.25	1.14	1.10	1.11	1.03	0.0240	<0.001	<0.001	0.19
Ammonia	mM	11.55	25.22	20.74	16.03	19.08	18.77	18.07	17.62	0.2569	<0.001	<0.001	0.003

(#) Doses are expressed as mg of fresh homogenate (6.89% of DM) added to each fermentation bottle.
T: incubation time; S: substrate; D: dose; DMD: dry matter digestibility; GP: Cumulated volume of gas produced; Total VFA: total volatile fatty acids.

caps and then an aerobically incubated in a water bath at $39 \pm 0.5\,°C$. Two sets of vials were incubated in duplicate: the fermentation of the first set was stopped after 8 h of incubation, the second one after 24 h. Duplicate vials containing no substrate and no homogenate were also prepared as blank controls. The overall incubation design was repeated twice, with a one week interval between experiments. Gas production was recorded at 2, 4, 6, 8, 14, 20 and 24 h by a high precision digital pressure gauge (LabDMM, AEP, Cognento, Italy), combined with releasing the gas produced after each measurement. Gas pressure readings were converted to volumes based on a pre-established volume/pressure calibration curve. The gas production was corrected for gas released from blanks and expressed as mL g^{-1} OM. At the end of the incubations, the serum vials were cooled in ice water to stop fermentation. The content was filtered on pre-tared crucibles (P2 porosity), oven dried at $103\,°C$ overnight and weighted to measure apparent dry matter digestibility. On the filtrate, pH was immediately measured potentiometrically and an aliquot of the remaining fluid was centrifuged at $3000 \times g$ for 10 min at $4\,°C$; 2 mL aliquots of the supernatant were transferred into tubes containing 1 mL of 0.12 M oxalic acid and frozen at $-20\,°C$ until VFA and NH3 analysis. The VFA were analysed as described by Minuti et al. (2014) and corrected for VFA concentrations recorded in blanks.

NH$_3$ was analysed using a clinical auto-analyser (ILAB 600, Instrumentation Laboratory, Lexington, MA) and the kit for urea nitrogen from the same company (code 0018255440).

Experiment 2: rumen degradation of the Aloe arborescens homogenate (in vivo trial)

Three rumen-cannulated dairy heifers, fed as stated in experiment 1, were used in a 3×3 Latin square design. The experimental treatments were: (1) control (1 L of water dosed through the rumen fistula); (2) Aloe arborescens homogenate (200 g head^{-1} d^{-1}) dispersed in 1 L of water and pulse dosed through the rumen fistula; (3) same dosage of dispersed Aloe homogenate, but delivered as an oral drench with a drenching bottle. Animals were dosed approximately 3 h after the morning meal. The experimental periods were separated by a one week washout interval, which was found in preliminary tests to be fully adequate for the complete disappearance of aloin from rumen content and blood circulation.

Ruminal fluid was collected at 0, 2, 4, 6, 12 and 24 h after Aloe arborescens administration to determine pH and concentration of aloin and aloe-emodin. At the same times blood samples were withdrawn from the jugular vein in 10 mL Li-heparin tubes (Vacuette, Greiner Bio-one, GmbH, Kremsmünster, Austria), immediately cooled in ice-water, delivered to the laboratory and processed as described in Calamari et al. (2016).

Experiment 3: absorption of aloin from the gut into the blood of dairy cows

Management of cows and diet

The experiment involved six multiparous, lactating and healthy Holstein dairy cows (244 ± 95 d in milk; 34.2 ± 13.0 kg d^{-1} milk yield; 22.4 ± 3.4 kg d^{-1} of dry matter intake. Data is expressed as mean $\pm$ SD), housed in an experimental tie-stall barn with controlled internal environmental conditions (16 h of light, temperature around $20\,°C$ and relative humidity around 65%). During the experiment the animals continued to be fed their usual a ration, formulated according to their requirements and composed of grass hay (2.8 ± 1.4 kg), alfalfa hay (2.7 ± 0.5 kg), corn silage (18.2 ± 3.3 kg) and concentrate (12.7 ± 3.0 kg). Forages were fed twice a day (0730 h and 1930 h), whereas concentrate was provided every three hours by an automatic system. The health status was regularly monitored during the experiment.

Cows were drenched with 200 g of Aloe arborescens homogenate, diluted to 1 L with water immediately before the morning forage meal. This dosage was chosen based on a previous in vivo trial on periparturient dairy cows (Trevisi et al. 2013). A few minutes before the administration of the Aloe arborescens homogenate, a fortified concentrate, containing 50000 IU g^{-1} of rumen-protected vitamin A, was offered to the cows, at a rate of 1% of their dry matter intake, in their cleaned manger and was rapidly and completely consumed by all the animals. Absorption of vitamin A from the gut increases the blood level of retinyl-palmitate. As vitamin A tends to remain stuck to the particles of concentrate, the kinetics of appearance for retinyl-palmitate in blood can be used to estimate the passage rate of concentrate from the rumen (Trevisi et al. 2007).

Blood samples were collected before morning feeding (0 h) and Aloe arborescens dosage and 2, 4, 6, 12, 24 h after dosage. The samples collected before and 24 h after Aloe arborescens administration were analysed to detect any possible acute effect due to the administration of the homogenate.

Analyses on plasma

The blood samples collected in experiments 2 and 3 were cooled in ice water immediately after bleeding.

Haematocrit was measured on a small aliquot of fresh blood and the remaining blood was spun for 15 min at 3520 × g at 4 °C and the resulting plasma was stored at −20 °C. The samples collected at 0 and 24 h from *Aloe arborescens* dosage (experiments 2 and 3) were analysed for parameters related to the energy status (glucose), inflammation (haptoglobin and ceruloplas-min,) and liver activity (bilirubin, aspartate transamin-ase, γ-glutamyl transferase, albumin and cholesterol). The analyses were carried out by an auto-analyser for chemical chemistry (ILAB 600, Instrumentation Laboratory, Lexington, MA) according to the methods previously described by Calamari et al. (2016).

Plasma retinyl-palmitate from all the samples of the experiment 3 was extracted with hexane and analysed by reverse-phase HPLC (Varian Inc., Palo Alto, CA) using a Spherisorb ODS-2, 3 μm in a 150 × 4.6 mm column (Alltech, Deerfield, IL) and an UV detector set at 325 nm. A methanol: tetrahydrofuran mixture (80:20 by volume) was used as mobile phase (Trevisi et al. 2007).

The aloin and aloe-emodin plasma content were analysed as described in the following paragraph.

Aloin and aloe-emodin assay

Aloe homogenates, rumen fluid and blood samples were analysed using liquid chromatography coupled to triple quadrupole mass spectrometry via an electro-spray ionization source (LC-ESI/MS/MS), as reported previously (Lucini et al. 2015). Briefly, samples were acquired in multiple reactions monitoring tandem mass spectrometry, preceded by reversed phase liquid chromatography.

Aloin was determined in duplicate for five different samples of leaf homogenate as well as for all the rumen fluid samples (2 g per each matrix), using the extraction method reported by Lucini et al. (2013). As far as aloin and aloe-emodin determination in plasma is concerned, samples (400 μL) were extracted in 1 mL of acidified methanol (1% formic acid), centrifuged (6 min at 3136 × g) and analysed using the same instrumental method as above.

The method was validated before sample analysis. For this purpose, recovery tests were carried out on plasma at 12, 20 and 50 μg kg^{-1} spike-in levels. Triplicate tests were carried out at each level, and then accuracy (as mean recovery) and precision (as relative standard deviation) were calculated. Validation parame-ters demonstrated that the method fits for our purpose (Table 2) although a quite strong ionic suppression (22–25% as compared to standards in solvent) forced the use of matrix-matched standards. Concerning accuracy, recoveries ranged from 70 to 99%, with a

mean value of 82%, whereas the precision as relative standard deviation was 13% (standard deviation 11%, $n = 9$). The dynamic range was linear (R$^2 > 0.99$) over three orders, up to a concentration of 500 ng L^{-1}.

Statistical analysis

Data from *in vitro* fermentations were processed by incubation time (8, 24 h) by ANOVA using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC; release 9.2) considering as fixed factors the time (8 and 24 h), the substrate (barley, alfalfa), the dose (0, 20, 100, 500) and the interactions between factors.

The concentrations of the plasma parameters from dairy cows before and 24 h after the Aloe administra-tion were processed through a one-way analysis of variance (GLM procedure, SAS Inst. Inc., Cary, NC; release 9.2) considering the time (0, 2, 4, 6, 12, 24 h) as a fixed factor.

Statistical significance was designated in all cases at $p < 0.05$, tendencies were declared at $p < 0.10$.

Results and discussion

Aloe arborescens *homogenate composition*

The homogenate of Aloe used in these experiments was composed by leaves from 1 to 3 years old plants and had a dry matter content of 6.89%. This homogen-ate contained (on dry matter basis): 13.41% CP, 1.74% ether extract, 24.10% NDFom and 18.40% ash. The remaining components of the homogenate were likely complex polysaccharides (e.g. β-polysaccharides, ace-mannans and aloerides; Hamman 2008; Pugh et al. 2001) and other substances (e.g. phenolics).

Aloin and aloe-emodin concentrations were meas-ured via LC-MS/MS in two samples from the same batch of homogenate (obtained from 18 plants). Aloin concentration was found to average 24.54 ± 0.18 g kg^{-1} of DM. Conversely, aloe-emodin was

Table 2. Accuracy and precision from aloin recovery tests ($n = 9$, triplicate determinations at three spiking levels) in bovine plasma.

Spiking level (μg L^{-1})	Amount recovered (μg L^{-1})	Recovery	Mean	SD
50	43	87%	81%	6%
50	37	75%		
50	40	80%		
100	72	72%	72%	2%
100	70	70%		
100	74	74%		
200	198	99%	96%	2%
200	191	95%		
200	190	95%		
Overall			82%	11%

not detectable in the leaves of our *Aloe arborescens*. The concentration of aloin and aloe-emodin may vary among different Aloe species (Hamman 2008; Di Luccia et al. 2013). In addition, breeding factors, sampling and post-harvest conditions can play an important role on the chemical characteristics of Aloe homogenates (Wang et al. 2010; Lucini et al. 2013). Thus, the negligible levels of aloe-emodin detected may be related to the plant cultivar and portion used, as well as to growth conditions. Moreover, physical–chemical or biological factors can quickly degrade *Aloe arborescens* homogenates (Pellizzoni et al. 2011). Storage at $-20\,^{\circ}C$ is an easy method to prevent degradation of the active molecules. In addition, the use of additives could interfere with the activity of the Aloe components and also with rumen fermentation. On this basis, the trials in this study were carried out using homogenates of *Aloe arborescens* stored at $-20\,^{\circ}C$ in 200 g aliquots and thawed at the time of use, in order to preserve as much chemical integrity as possible.

Aloe – rumen interactions (experiments 1 and 2)

The results concerning the effects of *Aloe arborescens* homogenate on *in vitro* rumen fermentations obtained in experiment 1 are reported in Table 1. As expected, *in vitro* fermentation parameters were significantly modified by the substrate. Incubating barley vs. alfalfa resulted in higher volumes of gas production (212 vs. 163 mL g^{-1}; $p < 0.01$) and VFA (86.3 vs. 80.8 mM; $p < 0.01$). VFA molar proportions were also affected ($p < 0.01$) by the substrate, as alfalfa leaded to a higher proportion of acetate but lower of propionate and butyrate. The addition of Aloe did not modify the fermentation parameters, except for the concentration of ammonia that was slightly but significantly reduced at the highest dose of Aloe ($p < 0.01$).

Calabrò et al. (2013) tested dried leaves from *Aloe arborescens* at doses up to 12% of the *in vitro* incubated dry matter of different substrates. Aloe did not influence the digestibility of the organic matter but increased gas production and, to a greater extent, total VFA yield. An increase in acetate and the tendency of propionate and butyrate to decrease were also recorded. The lower doses used in our trial, as well as the different technique used to obtain and manipulate the plant, can account for these differences. The Aloe homogenate used in our study did not demonstrate any adverse impact on the rumen fermentations, whereas it tended ($p = 0.10$) to increase the total VFA production and reduced the concentration of ammonia. Sirohi et al. (2009) reported a positive effect on *in vitro* dry matter digestibility using an acetone-extract

from leaves of *Aloe barbadensis*, but did not observe the same effect using methanol or water extracts, suggesting that the positive influence on digestibility is due to the hydrophobic component, likely contained in a higher amount in the derma.

Aloin was never detected in the rumen samples collected in experiment 2 from cannulated heifers after either oral or intra-cannula administration of *Aloe arborescens* homogenate, even at the first sampling time point (2 h after dosage). Aloin was detected in blood samples from the same animals, although randomly and at traces levels. For these reasons, data from this trial was not statistically processed. These results suggest that aloin was rapidly metabolised within the rumen, at least in high fibre diets. The bacterial degradation of aloin through cleavage of the C-glucoside residue that Che et al. (1991) hypothesised took place in the human intestine, may also occur in cattle rumen. Nevertheless, the escape to the omasum of unaltered small amounts of Aloe homogenate, available for further absorption as well as a direct and rapid absorption of aloin through the rumen wall can explain the small, random increases in blood aloin levels. Our data does not demonstrate a preference for either of these hypotheses.

Transfer of aloin into the plasma and effects on hematic profile (experiment 3)

The administration of the Aloe homogenate to lactating cows did not cause adverse effects on the health status of the animals (feed intake, milk yield, udder and foot health to rectal temperature) in the 48 h following administration. In the same period no cases of diarrhoea were observed, although laxative effects are often associated to *Aloe vera* intake (Capasso et al. 1998) and ascribed to the conversion of barbaloin into aloe emodin anthrone by intestinal bacteria. Feed intake was not statistically different between the day before and after Aloe administration. None of the metabolic parameters controlled in blood plasma showed statistical differences between pre (0 h) and post (24 h) Aloe administration and blood parameters remained within the reference values (Bertoni & Trevisi, 2013). The plasma retinyl-palmitate appeared at 1.5 ± 0.7 h and peaked at approximately 11 h after oral administration of the vitamin-A fortified concentrate, in agreement with the typical pattern observed in mid-lactating cows (Trevisi et al. 2007).

Aloe-emodin was never detected in the plasma samples, whereas aloin was. The pattern of the plasma aloin concentrations after the administration of *Aloe arborescens* homogenate is shown in Figure 1. Despite some

differences between animals, a similar trend was observed in all cases: aloin plasma levels were detectable as early as 2 h after administration, then increased to peak at 4 h after *Aloe arborescens* dosage, at an average concentration of $6.2 \pm 5.8\,\mu g\,L^{-1}$. After peaking, the concentration sharply declined at 6 h and then slowly declined to a mean value of $0.9 \pm 0.6\,\mu g\,L^{-1}$ at 24 h.

Our results show that aloin can be absorbed from the gut into the blood, as it became detectable in plasma after a single administration to lactating cows. Data on plasma aloin concentration showed a notably high variability between animals (Figure 1). This result may be due to the different transit time through the rumen for different animals, but an imperfect mixing of the dosed homogenate within the rumen digesta can also be hypothesised. To the best of our knowledge, only two studies, both on rats, have previously studied the absorption of anthraquinones (Park et al. 2008, Yan & Ma 2007). In both studies, approximately $12\,g\,\,kg^{-1}$ of live weight of anthraquinones were administered. The maximum aloin levels in the plasma measured in these studies were ten times higher than those observed here. These differences are not justified by only the lower amount of aloin dosed to dairy cows (7.6 mg of aloin kg^{-1} live weight), but suggest that aloin was largely metabolised in the bovine forestomachs. This is in agreement with the results obtained in experiment 2 on heifers where aloin disappeared extremely rapidly from the rumen content and only traces were detected in the plasma of these animals. In experiment 2, a slow rumen outflow rate can be hypothesised as the animals were fed at about maintenance, and this likely allowed for active degradation of the aloin. In experiment 3 with dairy cows, the oral drenching that delivered the homogeneate close to the reticulo-omasal orifice and the higher feeding levels likely provided th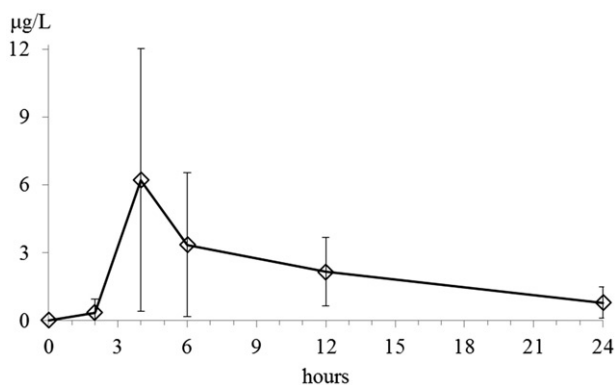e opportunity for aloin to partially escape rumen degradation. Differences in the diet between heifers and lactating cows may have also contributed to the different degradation ability of the rumen microbiota. Nevertheless, our data cannot exclude a quick and partial absorption of aloin directly from the rumen wall.

The peak aloin concentration in the plasma of lactating cows was reached later than in rat (Park et al. 2008), where the peak was recorded one hour after oral administration of b-D-glucuronidase and sulfatase aloin, but the following decrease was very similar in both species. This result can be likely explained by the differences in anatomy and physiology between the digestive apparatuses of monogastrics and ruminants. Furthermore, a comparison can be made involving the absorption kinetic of the rumen-protected vitamin A that was dosed concurrent with the Aloe homogenate. The vitamin in this form escapes rumen degradation and appears in plasma as retinyl-palmitate some hours after the meal (Trevisi et al. 2007). Aloin kinetic in comparison to retinyl-palmitate kinetic showed an anticipated peak in the plasma, suggesting a quicker rumen passage or a faster post-ruminal absorption. Aloin is soluble in water and likely follows the faster flow of liquids, whereas vitamin A administered in a form that remains associated with the solid components (Trevisi et al. 2007). Techniques that accelerate outflow from the rumen or protect *Aloe arborescens* components from the rumen degradation could be useful in allowing a higher aloin absorption from the gut.

Two main factors probably contributed to the rapid decline of aloin in blood observed after the concentration peak: hepatic conversion into its glucuronide derivative (Park et al. 2008) and a rapid metabolisation in the rumen, which we consider to be the most relevant explanation.

Although there is only a transient appearance of aloin in blood, the evidence that aloin can be transferred into plasma suggests possible systemic effects of *Aloe arborescens*, as recently hypothesised for dairy cows by Trevisi et al. (2013).

Conclusions

The homogenate of *Aloe arborescens* whole leaves did not negatively affect ruminal *in vitro* fermentations, blood parameters or health status in the short-term. Despite observable uptake, aloin seems to be rapidly transformed in the rumen. We cannot exclude its absorption through the rumen barrier, at least when the homogenate is orally drenched before the main meal into lactating cows with high feed intake and receiving a relatively low-fibre diet. Nevertheless, our

Figure 1. Aloin plasma concentration (mean $\pm$ standard deviation) in cows during the 24 h following oral administration of 200 g of *Aloe arborescens* homogenate.

results suggest for the first time that a significant portion of aloin is absorbed in the gut of lactating cows. Literature reported anti-inflammatory effects of *Aloe arborescens* in humans and monogastric animals. Our data suggest that Aloe might also have a nutraceutical value in dairy cows, although further studies are required and an optimal dose to be used *in vivo* must be defined.

Acknowledgements

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding information

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